SOME NUTRITIONAL REQUIREMENTS OF SPOILAGE MOLDS OF PINEAPPLE FRUIT

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ABSTRACT – The utilization of 6 carbon and 7 nitrogen sources by 6 pineapple fruit pathogens : *Ceratocystis paradoxa, Rhizopus stolonifer, R. oryzae* (soft rot spoilage molds), *Curvularia verruculosa, Penicillium claviforme* and *Aspergillus flavus* (dry rot spoilage molds) for their growth was determined. The dry rot molds only grew well on glucose and sucrose while the soft rot molds substantially utilized all the carbon sources. All the molds grew well on casein hydrolysate. The 2 inorganic nitrogen sources did not support the growth of the molds except *P. claviforme*. All the organic nitrogen sources supported good growth of *C. paradoxa* and *C. verruculosa*.

RÉSUMÉ – L'utilisation de 6 sources de carbone et de 7 sources d'azote a été déterminée pour la croissance de 6 agents d'altération des fruits d'ananas : *Ceratocystis paradoxa, Rhizopus stolonifer, R. oryzae* (responsables de la pourriture molle), *Curvularia verruculosa, Penicillium claviforme* et *Aspergillus flavus* (responsables de la pourriture sèche). Seuls les champignons de la pourriture sèche présentent une bonne croissance sur le glucose et le saccharose, tandis que les champignons de la pourriture molle ont utilisé substantiellement toutes les sources de carbone. Tous les champignons présentent une bonne croissance sur l'hydrolysat de caséine. Les 2 sources d'azote inorganique n'ont aucune influence sur la croissance des champignons, exception faite du *P. claviforme*. Toutes les sources d'azote organique donnent une bonne croissance du *C. paradoxa* et du *C. verruculosa*.

KEY WORDS : fruit spoilage molds, nutritional requirements, pineapple.

INTRODUCTION

The pineapple fruit is used as a supplement in the Nigerian diet. The edible portion, constituting approximately 60 % of the fruit (PURSEGLOVE, 1972) is basically of carbohydrate (glucose 9700-12000 mg per 100 mg fruit) and protein (3600-5000 mg per 100 g fruit) nature (DUCKWORTH, 1966). BHARGAVA (1974) reported the utilization of sucrose after hydrolysis, maltose and cellobiose through transglycosidation by 3 storage rot fungi : *Fusarium solani* (Mattius) Saccardo, *Botryodiplodia ananassae* and *Macrophomina phaseoli* (Maublanc) Ashby. The work of PATHAK (1971) showed that the mango fruit

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stem-end rot mold, *Diplodia natalensis* Pole Evans, did not grow well in nitrogen sources though the mold preferentially utilized cellulose and pectin for growth. Four isolates of *Botryodiplodia theobromae* Pat. utilized polysaccharides including starch and dextrin (SRIVASTAVA & TANDON, 1969). TERUI & HARADA (1970) reported that glucose, saccharose and pectin were the best sources of carbon for the brown rot organism, *Monilinia fructicola* (Winter) Honey.

The preferential utilization of the carbon and nitrogen sources by these various fruit spoilage molds might have an influence on their ability to produce the types of deterioration on their respective fruit as substrates. Carbon and nitrogen compounds have an unparallel role in the nutritional physiology of these fungi.

In an earlier study, it was shown that *C. paradoxa* produced spoilage of unripe pineapple fruit when certain concentrations of carbon and nitrogen infusions were applied on mature green fruits (ADISA, 1983). This mold causes soft spoilage with the production of watery fluid. Therefore the nutritional requirements of the dry and soft spoilage molds would be different. The 6 molds under investigation are soft and dry rot molds of the pineapple fruit. The utilization of some carbon and nitrogen sources for growth by these molds was investigated and the results obtained are presented in this paper.

MATERIAL AND METHODS

The fungi used – *Ceratocystis paradoxa* (Dade) C. Moreau (IMI 223309), *Rhizopus stolonifer* (Ehrenb. ex Fr.) Lind. (IMI 223284), *R. oryzae* Went & Prinsen Geerligs (IMI 223286), soft rot molds; *Curvularia verruculosa* Tandon & Bilgrami (IMI 223307), *Penicillium claviforme* Bainer (IMI 223298) and *Aspergillus flavus* Link ex Fr. (IMI 223288), dry rot molds – were reported as spoilage molds of pineapple fruits in Nigeria (ADISA & FAJOLA, 1982).

The effects of carbon and nitrogen sources on the growth of the fungi were investigated. The carbon sources used include D-glucose, sucrose, dextrin, starch (soluble potato), carboxymethylcellulose (Na salt, CMC) and pectin (apple grade 250). The nitrogen sources used were : DL-aspartic acid, L-asparagine, L-lysine, DL-glutamic acid, casein hydrolysate, ammonium sulphate and sodium nitrate.

The basal medium for these growth studies contained : D-glucose, 5000 mg; $MgSO_4.7H_2O$, 750 mg; distilled water, 1000 ml (pH 6.0). All salts were «Analar» grade (BDH Laboratories). This medium was stabilized with citric-acid-phosphate buffers before autoclaving at 121°C for 15 min. (for carbon sources) and 10 min. (for nitrogen sources). 25 ml of each medium was distributed into 250 ml flasks and the pH adjusted to 7.0 before autoclaving. For the effects of carbon and nitrogen sources requirements on mycelial production by fungi, D-glucose and the nitrogen source in the basal medium were respectively replaced by each of the carbon and nitrogen sources used to give 0.8 g carbon/l (OSO, 1974) and 0.485 g nitrogen/l (HASIJA & AGARWAL, 1978) except for starch,

dextrin, pectin and CMC which were added to give 20 g/l of basal and casein hydrolysate to give 5 g/l.

For inoculum, the fungi were cultivated on 2 % malt extract agar for 3 days. A 5 mm diameter of ■ fungus was inoculated in each flask and incubation was done at 25°C for 5 days without agitation. Inoculated basal medium served as control. The mycelium produced was determined by the dry weight method.

RESULTS

Glucose and sucrose generally supported the growth of all the fungi with *C. verruculosa* and *P. claviforme* producing the highest mycelia on them respectively (Table 1). Though starch and dextrin substantially gave good growth for all fungi, carboxymethylcellulose and pectin were not well utilized by *A. flavus*, *P. claviforme* and *C. verruculosa*. But *R. stolonifer*, *R. oryzae* and *C. paradoxa* grew well on the two polysaccharides (Table 1).

Carbon source	Quantitative production of mycelium (mg) by rot pathogens within specified pH ranges											
	Chaetopsella paradoxa		*Rhizopus stolonifer*		*Rhizopus oryzae*		*Penicillium verruculosum*		*Penicillium claviforme*		*Aspergillus flavus*	
	a	1	b	2	c	3	d	4	e	5	f	6
D-glucose	128.0±1.4*	5.8	94.0±0.8	6.0	78.4±1.6	5.8	140.6±1.3	6.5	130.1±1.1	5.6	68.0±1.1	5.6
Sucrose	119.6±1.1	6.0	103.8±1.3	6.0	73.9±1.0	7.0	118.5±1.6	6.8	134.8±0.6	5.5	61.2±0.5	5.8
Dextrin	104.3±0.8	6.0	85.1±0.6	7.3	83.3±1.2	6.0	86.5±0.8	6.3	50.8±0.7	6.0	54.0±1.5	5.3
Starch	98.6±0.3	5.7	66.7±1.0	7.0	70.0±1.0	6.5	98.5±1.0	6.6	41.6±0.6	6.8	47.6±1.7	6.8
CMC**	84.6±1.5	6.0	63.7±0.9	6.1	76.1±0.5	6.0	21.6±0.4	7.0	19.4±0.5	6.0	29.6±1.3	5.0
Pectin	96.7±1.9	5.5	67.9±1.2	7.2	66.5±1.4	6.5	30.5±1.2	7.0	23.0±1.3	6.0	22.1±0.6	5.7

a-f = dry weight mycelium in mg/25ml of medium of rot pathogens (minus control values); 1-6 = pH of filtrates after experiment; * = standard error; ** = Na salt of carboxymethyl cellulose.

Table 1 – Growth of 6 pineapple fruit rot pathogens in liquid media containing 6 carbon sources, after 5 days incubation at 25°C (Data are means of 5 replicates).

Tableau 1 – Croissance de 6 agents pathogènes de la pourriture de l'ananas, sur milieux liquides contenant 6 sources de carbone, après 5 jours d'incubation à 25°C (Moyennes de 5 répétitions).

Inorganic nitrogen sources did not give good growth for all fungi, however, they all recorded highest growth on casein hydrolysate (Table 2). *C. paradoxa* grew well on L-asparagine and L-glutamic acid. The *Rhizopus* spp. recorded some appreciable growth on L-lysine, DL-aspartic acid, L-asparagine, L-lysine and L-glutamic acid supported good growth of *C. verruculosa*. Except in casein hydrolysate, *P. claviforme* produced the highest mycelium in ammonium sulphate and sodium nitrate. *A. flavus* grew well only in DL-glutamic acid. The pH of the filtrates after the experiments ranged between 5.0 and 7.3 (for carbon sources) and 5.7 and 8.1 (for nitrogen sources). The production of high quantity of mycelia occurred both at low and high pH values.

Nitrogen source	Quantitative production of mycelium (mg) by rot pathogens within specified pH ranges											
	Chalara paradoxa		*Rhizopus stolonifer*		*Botryodiplodia theobromae*		*Ceratocystis paradoxa*		*Penicillium claviforme*		*Aspergillus niger*	
	a	1	b	2	c	3	d	4	e	5	f	6
DL-aspartic acid	49.5±0.9*	6.6	24.0±0.6	7.0	35.9±1.6	7.3	67.5±0.4	6.9	18.2±1.0	6.5	39.1±1.6	6.1
L-asparagine	102.6±1.2	6.3	28.6±0.4	7.1	29.3±1.1	7.3	49.6±1.5	5.8	26.0±1.0	7.0	23.2±1.3	6.9
L-lysine	63.8±1.1	6.5	49.3±1.7	6.8	60.0±0.7	6.8	81.4±1.0	5.7	33.7±0.9	6.4	31.6±1.1	6.7
L-glutamic acid	80.5±0.4	7.2	29.6±1.3	6.5	34.9±0.9	6.5	42.0±0.3	6.8	14.2±0.3	6.8	49.2±1.2	6.0
Casein hydrolysate	134.9±1.8	7.5	56.3±0.6	7.7	72.9±1.0	7.8	138.9±1.8	5.9	86.3±1.3	7.6	72.1±1.3	7.6
Ammonium sulfate	44.5±1.3	8.1	16.5±0.2	7.5	18.7±0.6	7.9	30.0±1.1	7.5	52.3±1.1	7.6	28.9±1.1	8.0
Sodium nitrate	27.9±1.5	7.3	14.2±1.5	7.1	27.9±1.3	6.3	23.9±0.7	6.2	49.6±1.1	7.4	30.3±1.8	6.8

a-f = dry weight mycelium in mg/25ml of medium of rot pathogens (minus control values);
1-6 = pH of filtrates after experiment; * = standard error.

Table 2 – Growth of 6 pineapple fruit rot pathogens in liquid media containing 7 nitrogen sources, after 5 days incubation at 25°C (Data are means of 5 replicates).

Tableau 2 – Croissance de 6 agents pathogènes de la pourriture de l'ananas, sur milieux liquides contenant 7 sources d'azote, après 5 jours d'incubation à 25°C (Moyennes de 5 répétitions).

DISCUSSION

The present study has indicated which of the carbon and nitrogen sources used are suitable for growth of the 6 pineapple fruit molds. Protein and glucose are known to be of high occurrence in the edible portion of the pineapple fruit (DUCKWORTH, 1966). All the organisms were able to grow and utilize the glucose and sucrose satisfactorily. Glucose was found to support good growth of *Aspergillus niger* (HASIJA & WOLF, 1969; SINGH & TANDON, 1971) while *Botryodiplodia theobromae* grew well on some monosaccharides and oligosaccharides (AHMED & HUQ, 1973). Both fungi are common fruit spoilage molds.

C. verruculosa, a dry rot mold of the pineapple fruit (ADISA & FAJOLA, 1982), utilized starch and dextrin quite satisfactorily during the present investigation. The other dry rot molds – *P. claviforme* and *A. flavus* – did not grow well on the two polysaccharides. On the other hand, *C. paradoxa*, *R. oryzae* and *R. stolonifer* grew well on all the carbon sources. They produce fruit spoilage of pineapple fruit with watery consistency (ADISA & FAJOLA, 1982). PATHAK (1971) reported good growth of a soft rot mold, *Diplodia natalensis* on starch, pectin and cellulose. *R. stolonifer* satisfactorily utilized pectin and cellulose sources (SPALDING, 1963). This observable difference in the preferential assimilation of carbon sources by these 2 groups (dry and soft molds) of spoilage organisms may indicate that those that produce dry deterioration may produce less complex enzymes for the spoilage or degradation of the food substances in pineapple fruit. The utilization of the more complex polysaccharides and the organic nitrogen sources by soft rot organisms certainly indicates the ability of the orga-

nisms to rely on more complex enzymes to degrade these substrates.

It is perhaps the ability of these fungi to grow on such complex substrates that makes them fast spoilage organisms, producing highly extensive biodeterioration of pineapple fruits. The fruits contain considerable quantity of pectin and cellulose materials. ADISA & FAJOLA (1982) observed that the effects of the spoilage on the fruits by the molds produced fermented fruits and the spoilage is accompanied by offensive odour. The production of aroma and flavour compounds by mould species has been reported by some workers. The formation of alcohols, esters, benzene derivatives, terpenes and specific metabolites by certain fungi as well as aroma characteristics of various species of fungi in culture have been discussed (LATRASSE & al., 1985). This odour might therefore be due to the degradative products produced from the breakdown of the nitrogen and carbon sources present in the pineapple fruits by the metabolites of the molds.

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